EXHIBIT B

invention. In light of the present disclosure, numerous embodiments within the scope of the claims will be apparent to those of ordinary skill in the art.

EXAMPLES

I. Treatment of NZB/W F$_1$ Mice with IFN-γ or with Anti-IFN-γ Monoclonal Antibodies: A Model for Systemic Lupus Erythematosus in Humans

The F$_1$ hybrids of autoimmune New Zealand Black (NZB) mice and the phenotypically normal New Zealand White (NZW) mouse strain develop severe systemic autoimmune disease, more fulminant than that found in the parental NZB strain. These mice manifest several immune abnormalities, including antibodies to nuclear antigens and subsequent development of a fatal, immune complex-mediated glomerulonephritis with female predominance, remarkably similar to systemic lupus erythematosus (SLE) in humans. Knight and Adams (1978), J. Exp. Med. 147, 1653.

In both the human and murine forms of the disease, a strong association with MHC gene products has been reported. HLA DR2 and HLA DR3 individuals are at a higher risk than the general population to develop SLE (Reinertsen et al. (1978), N. Engl. J. Med. 299, 515), while in NZB/W F$_1$ mice (H-$2^{/u}$), a gene linked to the h-2^{u} haplotype derived from the NZW parent contributes to the development of the lupus-like nephritis.

I.A. Treatment with IFN-γ: Effect on the Survival of NZB/W F$_1$ Mice

I.A.1. Treatment of Four Month Old Mice with IFN-γ

Two groups of 25 female NZB/W F$_1$ mice, four months of age, received recombinant IFN-γ or equivalent volumes of phosphate-buffered saline (PBS) over a period of three months. The mice were four months of age at the beginning of treatment. As an additional control group, 16 age- and sex-matched mice of the parental strain NZW were similarly treated with IFN-γ.

Murine IFN-γ manufactured in *E. coli* by recombinant technology according to the procedure of Gray and Goeddel was kindly provided by Dr. Michael Shepard, Genentech, Inc. The procedure of Gray and Goeddel (1983) for the production of IFN-γ using recombinant techniques is found in Proc. Natl. Acad. Sci. U.S.A. 80, 5842, and is hereby incorporated by reference herein. Each mouse was given interperitoneal injections of 5×10 units of IFN-γ or PBS 3 times weekly for three months.

FIG. 1 presents a graph showing the survival of NZB/W F$_1$ mice treated with IFN-γ. The closed circles represent NZB/W F$_1$ mice treated with IFN-γ; the open circles represent the age- and sex-matched NZB/W F$_1$ mice treated with PBS; and the open triangles represent the NZW parental strain treated with IFN-γ. The arrows indicate the time period of the treatment.

As seen in FIG. 1, death occurred at an earlier age in the group of NZB/W F$_1$ mice that received IFN-γ compared to the PBS control mice. The difference in survival between treated and control mice was statistically significant (p 0.001). In the IFN-γ-treated group, 75–80% of NZB/W F$_1$ mice were dead by 8 months. In contrast, in the PBS control NZB/W F$_1$ group, the mice had only begun to die at 8 months, and at 9.5 months 50% of this group of mice were still surviving. The lifespan of the NZW control group was not affected by the IFN-γ treatment.

I.A.2. Treatment of Mice of Different Ages with IFN-γ

The treatment protocol was as in I.A.1., except that treatment with IFN-γ was initiated at different ages, ranging between 2½ to 6 months. This treatment accelerated mortality when compared to age-matched control groups. When IFN-γ treatment started at 6½ months, using the same protocol. no significant difference in lifespan was observed between the group treated with IFN-γ and controls.

I.B. Treatment with Anti-IFN-γ Monoclonal Antibodies

Groups of age matched female mice were treated intraperitoneally with monoclonal anti-IFN-γ either at 2 mg×3 per week, or at 2 mg once per week; control groups of age-matched female mice were treated intraperitoneally with PBS, or with nonrelevant monoclonal antibody. All mice were four months old at the beginning of treatment.

DB-1 monoclonal anti-IFN-γ antibodies (DB-1) were purified from ascites fluid of Balb/c mice injected with 10^7 DB-1 hybridoma cells. DB-1 cells were obtained from the TNO Primate Center, the Netherlands. Antibodies were purified by 40% ammonium sulfate precipitation (twice) followed by chromatography on a DEAE-Sephacel column.

The ability of DB-1 to inhibit IFN-γ-induced Ia expression was assayed in vitro on the murine myelomonocytic cell line WEHI-3. Treatment of these cells with murine IFN-γ for 24–48 hours induces the expression of Ia antigens on their surface. King and Jones (1983), J. Immunol. 131, 315. Treatment of WEHI-3 cells with 10 U/ml of murine IFN-γ in the presence of DB-1 at 0.5 mg/ml caused inhibition of about 70% of Ia expression as detected with fluorescein-conjugated MK-D6 (anti-Ia^d) monoclonal antibody on a fluorescence-activated cell sorter (FACS IV).

I.B.1. Effect on the Survival of NZB/W F$_1$ Mice

FIG. 2 shows the improved survival rate of mice treated with DB-1. The closed triangles indicate mice treated with PBS; the open circles indicate mice treated with the nonrelevant monoclonal antibody; the closed squares indicate mice treated with DB-1 at 2 mg×3 per week; and the open squares indicate mice treated with DB-1 at 2 mg once per week.

As shown in FIG. 2, at the age of 11 months, 80–85% of the mice in both control groups were dead. In contrast, 95% of the mice in both DB-1-treated groups were alive at this time. There was no difference found between mice given weekly 2 mg injections of DB-1 compared to those receiving 2 mg antibody 3 times per week.

I.B.2. Effect on the Development of Significant Proteinuria

Proteinuria was measured colorimetrically by the use of Uristix (Miles Laboratories, Inc., Elkhart, Ind.). This produces an approximation of proteinuria as follows: trace, 10 mg/dl; 1+, 30 mg/dl; 2+, 100mg/dl; 3+, 300 mg/dl; and 4+, 1000 mg/dl. Measurements of proteinuria were performed by an observer who had no knowledge of the treatment given.

FIG. 3(A) shows the cumulative frequency of significant proteinuria resulting after treatment with IFN-γ (closed circles), PBS or nonrelevant monoclonal antibody (open circles), or monoclonal anti-IFN-γ (DB-1) either three times per week (closed squares) or once per week (open squares). There were no significant differences found between PBS versus nonrelevant antibody-treated animals; therefore, they are presented together as a single control group. In order to reflect more accurately the development of renal disease in all mice (alive and dead), a correction factor was introduced. Thus, each point reflects the current level of proteinuria as well as the last measurement of proteinuria in deceased mice. Arrows indicate the time period of the different treatments.

As seen in FIG. 3(A), the development of high grade proteinuria was significantly delayed by treatment of the mice with DB-1 and stimulated by treatment with IFN-γ. At 8 months the development of high grade proteinuria was not detected in the DB-1 mice, while approximately 30% of the control mice and approximately 80% of the IFN-γ treated mice had high-grade proteinuria. In addition, the onset of high-grade proteinuria was delayed five months by the DB-1 treatment. In fact, the onset of high-grade proteinuria was not detected until 3 months after the treatment with DB-1 had ceased, raising the possibility that continued treatment with DB-1 may have further prevented the development of high-grade proteinuria.

The delaying effect of DB-1 on high-grade proteinuria parallels its dramatic effect on survival, as shown supra.

I.B.3. Effect on the Appearance of Anti-DNA Antibodies

The presence of anti-DNA specific antibodies in NZB/W F_1 mice were determined by using a modification of a linked immunosorbent assay (ELISA) described by Zouali and Stollar (1986), J. Immunol. Methods 90, 105. More specifically, polystyrene microtiter plates were irradiated with UV germicidal lamps for 12 hours followed by incubation with 100 μl of nucleic acid solution (10 μ/ml) in PBS, for 2 hours at room temperature (RT). Serum samples were incubated for 1 hr at RT. Bound antibodies were revealed with peroxidase-conjugated goat anti-mouse IgG (Tago, Inc., Burlingame, Calif.). Absorbance at 405 nm was read with a multiscan automatic spectrophotometer (Dynatech).

FIG. 3(B) shows the effect of treatment with DB-1 and with IFN-γ, as compared to controls, on the appearance of anti-DNA antibodies. The open bars represent the mean age at which anti-DNA antibodies were detected in the various groups, while dashed bars represent the mean age at which peak levels of anti-DNA antibody occurred. Note: at the age when anti-DNA antibody was detected, the difference between the PBS and IFN-γ-treated groups is not significant, while the difference between PBS and DB-1 treated groups is significant (p 0.05). The differences in age at peak levels between PBS-treated versus IFN-γ or DB-1-treated groups are significant (p 0.005 and p 0.0005, respectively).

As shown in FIG. 3(B), treatment with DB-1 delayed both the age at which anti-DNA antibodies were first detected and the age at which maximum levels of anti-DNA antibodies appeared. In both the IFN-γ-treated and the control mice, anti-DNA antibodies were first detected at about 5 months of age, as compared to between 6 and 7 months for the DB-1-treated mice. Maximum levels of DNA antibodies were seen at approximately 11 months in the DB-1-treated mice, whereas the appearance of maximum levels was at approximately 7 months and 8 months for the IFN-γ-treated and control mice, respectively.

II. Treatment of SJL/J Female Mice Immunized with Acetyl Choline Receptor Protein (AcChoR), with Anti-IFN-γ Antibodies: A Model System for Myasthenia Gravis in Humans

Myasthenia gravis is one of several human autoimmune diseases linked to HLA-D. Safenberg et al. (1978), Tissue Antigens 12, 136; McDevitt and Engelman (1977) Arth. Rheum. 20, 59. In myasthenia gravis, antibodies to the acetyl choline receptors (AcChoR) impair neuromuscular transmission by mediating loss of AcChoR in the postsynaptic membrane. Kao and Drachman (1977), Science 196, 527; Heinemann et al. (1977), Proc. Natl. Acad. Sci. U.S.A. 74, 3090.

SJL/J female mice are a model system for human myasthenia gravis. Waldor et al. (1983) 80, 2713. In these animals, experimental autoimmune myasthenia gravis (EAMG) is induced by immunizing the mice with soluble AcChoR protein from another species. Susceptibility to EAMG is linked in part to the MHC and has been mapped to the I region within H-2. Christadoss et al. (1979), J. Immunol. 123, 2540.

II.A. Methods of Inducing Antibodies to AcChoR and of Inducing Clinical EAMG

AcChoR protein is purified from *Torpedo californica* and assayed according to the method of Waldor et al. (1983). Proc. Natl. Acad. Sci. U.S.A., 2713, which is hereby incorporated by reference.

EAMG is induced in SJL/J mice by immunization with AcChoR. More specifically, emulsified AcChoR, 15 μg in complete Freund adjuvant is injected intradermally among six sites on the back, the hind foot pads, and the base of the tail. Animals are reimmunized with this same regimen 4 weeks later.

II.B. Treatment of AcChoR Immunized SJL/J mice with Anti-IFN-γ Antibodies

Treatment of the mice is as follows. Animals are divided into three matched groups. Treatment with anti-IFN-γ antibody is as follows: group 1 is treated prior to the initial immunization with AcChoR, group 2 is treated between the first and second immunization, and group 3 is treated immediately after the second immunization. Control groups of animals are treated according to this schedule, substituting PBS or irrelevant monoclonal antibody for anti-IFN-γ antibody.

II.B.1. Effect of the Treatment on the Titer of Anti-AcChoR Antibodies

Anti-AcChoR antibody levels are measured by a microtiter ELISA assay as described in Waldor et al., supra. More specifically, The standard reagent volume is 50 μl per well. Reagents are usually incubated in the wells for 2 hr at RT. Five Vg of AcChoR diluted in bicarbonate buffer, pH 9.6, is added to each well. After incubation with AcChoR, the plates are rinsed four times with a wash solution consisting of phosphate-buffered saline containing 0.05% Tween and 0.05% NaN_3. Mouse sera are diluted in 0.01M PBS (pH 7.2), 1.5 mM $MgCl_2$, 2.0 mM 2-mercaptoethanol, 0.05% Tween-80, 0.05% NaN_3 (P-Tween buffer) and incubated on the plate. After the plate is washed, β-galactosidase-conjugated sheep anti-mouse antibody diluted in P-Tween buffer is added to each well. After a final washing, the enzyme substrate, p-nitrophenyl-galactopyranoside is added to the plate, and the degree of substrate catalysis is determined from the absorbance at 405 nm after 1hr.

Anti-AcChoR antibodies are expected to be present in the immunized untreated mice as compared to nonimmunized mice. Treatment with anti-IFN-γ is expected to significantly reduce the titer of anti-AcChoR antibodies. Mice treated with PBS and with an irrelevant monoclonal antibody are expected to have titers equal to that in immunized untreated mice.

II.B.2. Effect on Clinical EAMG

The effect of treatment with anti-IFN-γ on clinical EAMG is assessed. Myasthenia symptoms include a characteristic hunched posture with drooping of the head and neck, exaggerated arching of the back, splayed limbs, abnormal walking, and difficulty in righting. Mild symptoms are present after a standard stress test involving swimming. Berman and Patrick (1980), J. Exp. Med. 151, 204. Weakness is ameliorated (unless the mice are moribund) within 5–10 minutes of administration of neostigmine bromide (0.0375 mg/kg) and atropine sulfate (0.015 mg/kg) intraperitoneally.

Treatment of AcChoR-immunized mice with anti-IFN-γ antibody is expected to prevent or ameliorate the clinical symptoms of EAMG.